Mechanisms of Resistance to Macrolides and Lincosamides: Nature of the Resistance Elements and Their Clinical Implications

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Resistance to macrolides and lincosamides is increasingly reported in clinical isolates of gram-positive bacteria. The multiplicity of mechanisms of resistance, which include ribosomal modification, efflux of the antibiotic, and drug inactivation, results in a variety of phenotypes of resistance. There is controversy concerning the clinical relevance of in vitro macrolide resistance. Recent data, however, have shown that eradication of bacteria correlates with clinical outcome of acute otitis media in children and that macrolide therapy results in delayed eradication of macrolide-resistant pneumococci. These results support the need for in vitro detection of macrolide resistance and correct interpretation of susceptibility tests to guide therapy.

Macrolides have been known for >5 decades, and, since the introduction of erythromycin into therapy, a number of these molecules have been developed for clinical use. For years, these antibiotics have represented a major alternative to the use of penicillins and cephalosporins for the treatment of infections due to gram-positive microorganisms (mostly β-hemolytic streptococci and pneumococci); however, the worldwide development of macrolide resistance with wide variations, according to both the country and the bacterial species, has sometimes constrained to limit the use of these antibiotics to certain indications. Although the evolution of the macrolide class has been marked, in the 1990s, by the development of semisynthetic macrolides with improved pharmacokinetics and tolerability, these new derivatives have proved unable to overcome erythromycin resistance. By contrast, our increasing knowledge of the molecular mechanisms of resistance to macrolides has led to the pharmaceutical industry’s design of derivatives, such as the ketolides, that have activity against certain types of erythromycin-resistant organisms [1]. The data on these antimicrobials that are in development will not be discussed in this review, which is devoted to commercially available drugs. In addition, although certain mechanisms of resistance to macrolides affect streptogramins, these antimicrobials also will not be discussed. Other aspects of macrolide resistance are the multiplicity of resistance mechanisms and the diversity in phenotypic expression of several of these mechanisms. These aspects render it difficult to correctly interpret the in vitro susceptibility tests and, ultimately, the correct therapeutic use of this class of antibiotics. The present review aims to describe the genetic determinants and the practical implications of the resistance mechanisms of clinically important pathogens.

MACROLIDES, LINCOSAMIDES, AND THEIR SPECTRUM OF ACTIVITY

Macrolide and lincosamide antibiotics are chemically distinct but share a similar mode of action. They have a spectrum of activity limited to gram-positive cocci (mainly staphylococci and streptococci) and bacilli, to gram-negative cocci, and intracellular bacteria (Chlamydia and Rickettsia species). Gram-negative bacilli are generally resistant, with some important exceptions (i.e., Bordetella pertussis, Campylobacter, Chlamydia, Helicobacter, and Legionella species).

Macrolides are composed of ≥2 amino or neutral sugars attached to a lactone ring of variable size. Commercially available macrolides have a 14-membered (clarithromycin, dirithromycin, erythromycin, and roxithromycin) or 15-membered...
(azithromycin) lactone ring. Sixteen-membered ring macrolides (josamycin, midecamycin, miocamycin, rokitamycin, and spiramycin) are available in certain countries or in veterinary practice (tylosin). Lincosamides (clindamycin and lincomycin) are devoid of a lactone ring.

MECHANISMS OF ACQUISITION OF RESISTANCE TO MACROLIDES AND LINCOSAMIDES

Bacteria resist macrolide and lincosamide antibiotics in 3 ways: (1) through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, (2) through efflux of the antibiotic, and (3) by drug inactivation. These mechanisms have been found in the macrolide and lincosamide producers, which often combine several approaches to protect themselves against the antimicrobial that they produce. In pathogenic microorganisms, the impact of the 3 mechanisms is unequal in terms of incidence and of clinical implications. Modification of the ribosomal target confers broad-spectrum resistance to macrolides and lincosamides, whereas efflux and inactivation affect only some of these molecules.

RIBOSOMAL METHYLATION

In 1956, soon after the introduction of erythromycin into therapy, resistance emerged in staphylococci [2]. Biochemical studies indicated that resistance was caused by methylation of the ribosomal target of the antibiotics, which leads to cross-resistance to macrolides, lincosamides, and streptogramins B, the so-called MLSB phenotype [2]. Subsequently, the MLSB phenotype encoded by a variety of erm (erythromycin ribosome methylase) genes was reported in a large number of microorganisms [3]. The first erythromycin-resistant strains of streptococci were reported in the United Kingdom in 1959 and in North America in 1967 [4, 5]. So far, ribosomal methylation remains the most widespread mechanism of resistance to macrolides and lincosamides.

In pathogenic bacteria, Erm proteins dimethylate a single adenine in nascent 23S rRNA, which is part of the large (50S) ribosomal subunit [2]. The A2058 residue is located within a conserved region of domain V of 23S ribosomal RNA, which plays a key role in the binding of MLSB antibiotics. As a consequence of methylation, binding of erythromycin to its target is impaired. The overlapping binding sites of macrolides, lincosamides, and streptogramins B in 23S rRNA account for cross-resistance to the 3 classes of drugs. A wide range of microorganisms that are targets for macrolides and lincosamides, including gram-positive species, spirochetes, and anaerobes, express Erm methylases.

Nearly 40 erm genes have been reported so far [3]. In pathogenic bacteria, these determinants are mostly borne by plasmids and transposons that are self-transferable. A nomenclature system has been devised to avoid increasing complexity in designation. erm genes with deduced amino acid sequence identity of <80% have different letter designations. This new nomenclature distinguishes 21 classes of erm genes and as many corresponding Erm proteins. Four major classes are detected in pathogenic microorganisms: erm(A), erm(B), erm(C), and erm(F) [2, 3]. erm(A) and erm(C) typically are staphylococcal gene classes. erm(B) class genes are mostly spread in streptococci and enterococci, and the erm(F) class genes in Bacteroides species and other anaerobic bacteria [3]. In addition to the erm(B) genes, the ermTR genes, which are now considered a subset of the erm(A) class on the basis of sequence homology, can be detected in β-hemolytic streptococci [3]. That each class is relatively specific—but not strictly confined—to a bacterial genus, reflects easy gene exchange.

DIVERSITY IN MLSB RESISTANCE EXPRESSION

Expression of MLSB resistance can be constitutive or inducible. In inducible resistance, the bacteria produce inactive mRNA that is unable to encode methylase. The mRNA becomes active only in the presence of a macrolide inducer. By contrast, in constitutive expression, active methylase mRNA is produced in the absence of an inducer. Induction is related to the presence of an attenuator upstream from the structural erm gene for the methylase. Induction occurs posttranscriptionally, according to the model of translation attenuation in the case of the erm(C) (a staphylococcal determinant) and also probably in the case of the erm(A) and erm(B) determinants [6]. The presence of an inducer leads to rearrangements of mRNA, which allow ribosomes to translate the methylase coding sequence. The strains harboring an inducible erm gene are resistant to the inducers but remain susceptible to noninducer macrolides and lincosamides. The pattern of macrolide inducers depends on the erm gene or, more precisely, on the structure of the attenuator controlling the gene expression. Because the structure of the attenuator differs in each class or subclass of erm gene, different patterns of MLSB-inducible resistance are observed. The genetic background or bacterial host also plays a role in the specificity of induction, possibly in relation to differences in ribosomal structure or methylase expression. In practice, however, the preferential distribution of erm genes in certain bacterial species leads us to consider only a few major phenotypes of inducible MLSB resistance characterized in staphylococci and streptococci/enterococci.

Therefore, the diversity of inducible macrolide resistance may lead to complex phenotypes. By contrast, constitutive produc-
Expression of MLS\textsubscript{B} resistance in staphylococci. The \textit{erm}(A) and \textit{erm}(C) determinants are predominant in staphylococci [7]. The \textit{erm}(A) genes are mostly spread in methicillin-resistant strains and are borne by transposons related to \textit{Tn}554, whereas \textit{erm}(C) genes are mostly responsible for erythromycin resistance in methicillin-susceptible strains and are borne by plasmids. The resistance phenotypes conferred by inducible expression of both determinants are similar and are characterized by dissociated resistance to MLS\textsubscript{B} antibiotics because of differences in the inducing capacity of the antibiotics. The strains are resistant to 14- and 15-membered ring macrolides, which are inducers. By contrast, 16-membered ring macrolides, commercially available lincosamides, and streptogramins B that are not inducers remain active (table 1). In disk-diffusion tests, a D-shaped zone caused by induction of methylase production by erythromycin can be observed when a disk of erythromycin is placed near a disk of clindamycin or any noninducer 16-membered ring macrolide.

The use of clindamycin (or of a noninducer macrolide) for the treatment of an infection due to an inducibly resistant strain of \textit{Staphylococcus aureus} is not devoid of risk. Constitutive mutants can be selected in vitro at frequencies of $\sim 10^{-7}$ cfu in the presence of these antibiotics. Bacterial inocula exceeding $10^7$ cfu can be found in mediastinitis and in certain lower respiratory tract infections. The risk to patients is illustrated by reports of selection of constitutive mutants during the course of clindamycin therapy administered to patients with severe infections due to inducibly erythromycin-resistant \textit{S. aureus} [8, 9]. If the staphylococcal inoculum at the site of infection is higher, the risk for selection of a constitutive mutant should be higher. In fact, clinical isolates that are constitutively resistant to MLS\textsubscript{B} antibiotics are widespread, particularly in methicillin-resistant strains.

Expression of MLS\textsubscript{B} resistance in streptococci and enterococci. The spread of \textit{erm} genes belonging to the \textit{erm}(B) class and, rarely, to the \textit{erm}(TR) subset of the \textit{erm}(A) class accounts for the vast majority of resistance caused by ribosomal methylation in streptococci and enterococci. Inducible expression of these genes gives rise to a large variety of phenotypes differing from that of staphylococci; these phenotypes include high- or low-level resistance to erythromycin with susceptibility or resistance to clindamycin. The phenotypes and their correlation with the genotypes are still far from being understood. Inducibly expressed \textit{erm}(B) genes are present in a variety of streptococcal species, including \textit{β}-hemolytic streptococci, oral streptococci, \textit{Streptococcus pneumoniae}, and enterococci. Most members of the MLS\textsubscript{B} group, including clindamycin and 16-membered macrolides, are inducers at various degrees of ErmB methylase production [10]. In inducibly resistant \textit{S. pneumoniae}, this feature, combined with the production of various basal levels of enzyme—probably in relation to a relaxed control of methylase synthesis by the \textit{erm}(B) attenuator, might explain, at least in part, the complexity of phenotypes (table 1) [11, 12]. It has been shown, by induction studies including fusions of attenuators with a reporter gene, that the MLS\textsubscript{B} phenotype characterized by high-level cross-resistance to macrolides and lincosamides.

### Table 1. Phenotypes and genotypes of macrolide resistance resulting from due to ribosomal methylation, drug efflux, or drug inactivation in gram-positive cocci.

<table>
<thead>
<tr>
<th>Species, mechanism</th>
<th>Gene class</th>
<th>Phenotype designation</th>
<th>14- or 15-Md</th>
<th>16-Md</th>
<th>Cli</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Staphylococcus} species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribosomal methylation</td>
<td>\textit{erm}</td>
<td>MLS\textsubscript{B} inducible</td>
<td>R</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLS\textsubscript{B} constitutive</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Macrolide efflux</td>
<td>\textit{msr}(A)</td>
<td>MS\textsubscript{B}</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Lincosamide inactivation</td>
<td>\textit{lnu}(A)</td>
<td>L</td>
<td>S</td>
<td>S</td>
<td>S\textsuperscript{a}</td>
</tr>
<tr>
<td>\textit{Streptococcus} and \textit{Enterococcus} species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribosomal methylation</td>
<td>\textit{erm}</td>
<td>MLS\textsubscript{B} inducible</td>
<td>R or I</td>
<td>R or I or s</td>
<td>R or I or s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLS\textsubscript{B} constitutive</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Efflux</td>
<td>\textit{mef}(A)</td>
<td>M</td>
<td>R</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>\textit{Enterococcus faecium}</td>
<td>\textit{lnu}(B)</td>
<td>L</td>
<td>S</td>
<td>S</td>
<td>S\textsuperscript{a}</td>
</tr>
<tr>
<td>Lincosamide inactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textbf{NOTE.} Cli, clindamycin; 14-Md, 14-membered ring macrolides (clarithromycin, dirithromycin, erythromycin, and roxithromycin); 15-Md, 15-membered macrolide (azithromycin); I, intermediate resistance; MLS\textsubscript{B}, macrolides, lincosamides, and streptogramins B; L, lincosamides; MS\textsubscript{B}, macrolides and streptogramins B; M, 14–15-membered macrolides; Md, membered; R, resistant; S, susceptible; s, susceptible in vitro but risk of selection of constitutive mutants in vivo; 16-Md, 16-membered ring macrolides (josamycin and spiramycin).

\textsuperscript{a} Diminished bactericidal activity.
linosamides, which is commonly detected in pneumococci, is frequently inducible [11, 12, 13]. Similar to the case of the constitutive phenotype, no macrolide or lincosamide can be used. Other strains of S. pneumoniae that contain erm(B) are apparently susceptible to clindamycin while being resistant to the 14-, 15-, and 16-membered macrolides. The occurrence of an antagonism between erythromycin and clindamycin, as made obvious by the double-disk diffusion test, indicates inducible production of methylase. Again, the use of clindamycin should be discouraged. In Streptococcus pyogenes, the phenotypes conferred by erm(B) are similar. Some erythromycin-resistant strains that apparently are susceptible to clindamycin express, by disk diffusion, a zonal resistance characterized by regrowth near the disk of clindamycin after extended incubation. The erm(TR) gene is spread in β-hemolytic streptococci and has been found in a single strain of S. pneumoniae [14, 15]. Presence of the gene mostly results in inducible erythromycin resistance expressed at low (MIC, 1–8 μg/mL) or high (MIC, >128 μg/mL) levels, whereas 16-membered macrolides and clindamycin remain apparently active. Antagonism between erythromycin and clindamycin is also observed. Again, constitutive MLSb resistance, irrespective of the erm gene class, leads to cross-resistance between macrolides and lincosamides.

AN EMERGING MECHANISM: TARGET MUTATION

In vitro selection of Escherichia coli mutants that are highly resistant to erythromycin has been of considerable value for characterization of the binding site of this antibiotic to the ribosome. The clinical importance of this mechanism was only recently recognized with identification of mutations at either A2058 or A2059 in domain V of rRNA; A2058 and A2059 confer MLSb and ML resistance, respectively [16]. Depending on the species, bacteria possess from 1 to several rrr operons encoding 23S rRNA. In general, the mutations are observed in pathogens with 1 or 2 rrr copies, often with each copy carrying the mutation. This mechanism is responsible for clarithromycin resistance in the vast majority of, if not all, strains of Mycobacterium avium and Helicobacter pylori [16]. Similar mutations have also been reported in Treponema pallidum and Propionibacterium species. Clinical strains and laboratory mutants have recently been identified in S. pneumoniae, which harbors 4 rrr copies [17].

Mutations in ribosomal proteins L4 and L22 that confer erythromycin resistance have been documented in laboratory and clinical isolates of S. pneumoniae [17]. The changes are clustered in a highly conserved sequence of L4 and confer resistance to macrolides but not to clindamycin. Although these types of resistance are considered, by definition, to be non-transferable, the ability displayed by pneumococci to acquire extrinsic genes easily by transformation followed by homologous recombination might then lead to spread.

The prevalence and clinical importance of the pneumococcal mutants are not known. In particular, the in vivo conditions that lead to selection of mutant strains have not been studied. Because attention has been brought on these new types of resistance only recently, however, we believe that, so far, their importance has been underestimated.

ANTIBIOTIC EFFLUX

In gram-negative bacteria, chromosomally encoded pumps contribute to intrinsic resistance to hydrophobic compounds, such as macrolides. The pumps often belong to the resistance/nodulation/division family composed of proteins with 12 membrane-spanning regions. In gram-positive organisms, acquisition of macrolide resistance by active efflux is caused by 2 classes of pumps, members of the ATP-binding-cassette (ABC) transporter superfamily and of the major facilitator superfamily (MFS).

To date, the only efflux proteins conferring acquired macrolide resistance characterized in Staphylococcus species are ABC transporters encoded by plasmidborne msr(A) genes [18]. The msr(A) resistance determinant was originally detected in Staphylococcus epidermidis, and, since then, it has been found in a variety of staphylococcal species, including S. aureus. ABC transporters require ATP to function and are usually formed by a channel composed of 2 membrane-spanning domains and 2 ATP-binding domains located at the cytosolic surface of the membrane.

The msr(A) gene encodes a protein with 2 ATP-binding domains characteristic of ABC transporters. The nature of the transmembrane component of the MsrA pump remains unknown. The efflux system appears to be multicomponent in nature, involving msr(A) and chromosomal genes to constitute a fully operational efflux pump that has specificity for 14- and 15-membered macrolides and type B streptogramins (the MSb phenotype) [18]. The resistance is inducibly expressed. Erythromycin and other 14- and the 15-membered macrolides are inducers, whereas streptogramins B are not. Therefore, the strains are resistant to streptogramins B only after induction with erythromycin. Clindamycin is neither an inducer nor a substrate for the pump, and thus the strains are fully susceptible to this antimicrobial (table 1).

As expected, constitutive mutants are resistant to both erythromycin and streptogramins B but remain fully susceptible to clindamycin. This phenotype can be easily distinguished from the MLSb-inducible phenotype by use of the double-disk diffusion test, which shows a lack of interaction between erythromycin and clindamycin. This determinant is common in coagulase-negative staphylococci and increasingly is found in

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Table 2. Macrolide resistance among *Streptococcus pneumoniae* isolates, by region or country.

<table>
<thead>
<tr>
<th>Region, country</th>
<th>Site(s)</th>
<th>Year</th>
<th>Origin of sample</th>
<th>% PDSP</th>
<th>Intermediate or resistant to erythromycin (%)</th>
<th>Intermediate or resistant to Clι (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All strains</td>
<td>Penicillin susceptible</td>
<td>Penicillin intermediate</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>30 medical centers</td>
<td>1994–95</td>
<td>Various</td>
<td>23.6</td>
<td>10.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>34 medical centers</td>
<td>1997–98</td>
<td>Various</td>
<td>29.5</td>
<td>19.2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>33 medical centers</td>
<td>1999–2000</td>
<td>Various</td>
<td>34.2</td>
<td>26.2</td>
<td>6.1</td>
<td>42.8</td>
</tr>
<tr>
<td>USA</td>
<td>163 medical centers</td>
<td>1997–98</td>
<td>Various</td>
<td>12.3 (RS)</td>
<td>20.9*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>163 medical centers</td>
<td>1998–99</td>
<td>Various</td>
<td>14.7 (RS)</td>
<td>22.7*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>8 states</td>
<td>1996</td>
<td>Blood, CSF</td>
<td>21</td>
<td>11</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>1996</td>
<td>Blood, CSF</td>
<td>25</td>
<td>15</td>
<td>3.2</td>
<td>35.1</td>
<td>61.3</td>
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<td>96 laboratories</td>
<td>1996–97</td>
<td>Various</td>
<td>36.5</td>
<td>16</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>1 center, Atlanta area</td>
<td>1994–99</td>
<td>Blood, CSF</td>
<td>34</td>
<td>23</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>USA</td>
<td>26–28 laboratories</td>
<td>1997–99</td>
<td>Various</td>
<td>14 (RS)</td>
<td>17.7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Canada</td>
<td>5–8 laboratories</td>
<td>1997–99</td>
<td>Various</td>
<td>6.8 (RS)</td>
<td>10.4</td>
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<td>NS</td>
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<td>Canada</td>
<td>18 medical centers</td>
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<td>RTIs</td>
<td>21.2</td>
<td>9.3</td>
<td>4.4</td>
<td>21.1</td>
</tr>
<tr>
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<td>Various countries</td>
<td>10 laboratories</td>
<td>1997–99</td>
<td>Various</td>
<td>11.7</td>
<td>10.5</td>
<td>NS</td>
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<tr>
<td>Brazil</td>
<td>5 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>22.9</td>
<td>4.8*</td>
<td>2.6*</td>
<td>11.9*</td>
</tr>
<tr>
<td>Europe</td>
<td>Various countries</td>
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<td>1997–98</td>
<td>Various</td>
<td>27.4</td>
<td>19.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Various countries</td>
<td>20 hospitals</td>
<td>1998</td>
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<td>3.2–53.3</td>
<td>1–47.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
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<td>Various</td>
<td>10.4 (RS)</td>
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<tr>
<td>Belgium</td>
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<td>Various</td>
<td>2.8</td>
<td>11.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
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<td>7 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>66.5</td>
<td>58.4*</td>
<td>17.6*</td>
<td>69.4*</td>
</tr>
<tr>
<td>France</td>
<td>21 regional registries</td>
<td>1999</td>
<td>Adults; children</td>
<td>40; 53</td>
<td>48; 65</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Germany</td>
<td>8 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>7.8</td>
<td>9.9*</td>
<td>6.9*</td>
<td>35*</td>
</tr>
<tr>
<td>Italy</td>
<td>7 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>16.8</td>
<td>24.3*</td>
<td>21.4*</td>
<td>34.1*</td>
</tr>
<tr>
<td>Italy</td>
<td>Several hospitals</td>
<td>1997–98</td>
<td>Blood, CSF</td>
<td>5.4</td>
<td>29.3</td>
<td>NS</td>
<td>NS</td>
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<td>1999</td>
<td>Various</td>
<td>24.7</td>
<td>13.8</td>
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<td>NS</td>
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<tr>
<td>Spain</td>
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<td>1996–97</td>
<td>Various</td>
<td>60</td>
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<td>51.3</td>
</tr>
<tr>
<td>Spain</td>
<td>8 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>65.6</td>
<td>37.8*</td>
<td>15–5*</td>
<td>54.2*</td>
</tr>
<tr>
<td>UK</td>
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<td>1997–98</td>
<td>Various</td>
<td>10.8</td>
<td>8.7*</td>
<td>6–5*</td>
<td>31.6*</td>
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<td>8.9</td>
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<td>10.5</td>
<td>29.8</td>
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<td>Asia-Pacific region</td>
<td>Various countries</td>
<td>17 laboratories</td>
<td>1997–99</td>
<td>Various</td>
<td>17.8</td>
<td>38.6</td>
<td>NS</td>
</tr>
<tr>
<td>Japan</td>
<td>6 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>54.1</td>
<td>67.9*</td>
<td>48*</td>
<td>78.1*</td>
</tr>
<tr>
<td>China</td>
<td>4 hospitals</td>
<td>1997–98</td>
<td>Various</td>
<td>16.9</td>
<td>74.2*</td>
<td>72.8*</td>
<td>64.7*</td>
</tr>
<tr>
<td>Taiwan</td>
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<td>1996</td>
<td>Various</td>
<td>53.7</td>
<td>71.4</td>
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<td>NS</td>
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<tr>
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<td>Shangai</td>
<td>1999</td>
<td>Various</td>
<td>53</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
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<td>1996–97</td>
<td>Various</td>
<td>22.4</td>
<td>52.6</td>
<td>NS</td>
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<tr>
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<td>1996–97</td>
<td>Various</td>
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<td>4.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Senegal</td>
<td>1 laboratory</td>
<td>1996–97</td>
<td>Various</td>
<td>61.7</td>
<td>11.4</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Tunisia</td>
<td>1 laboratory</td>
<td>1996–97</td>
<td>Various</td>
<td>30.4</td>
<td>32.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>South Africa</td>
<td>8 provinces</td>
<td>1992–96</td>
<td>Blood, CSF</td>
<td>18</td>
<td>3</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. Clι, clindamycin; NS, data not shown in the publication; PDSP, pneumococci with diminished susceptibility to penicillins (MIC of penicillin G, >0.12 mg/L); RS, resistant strains; Ref., reference; RTIs, respiratory tract infections.

a Percentage of strains intermediate or resistant to azithromycin.

methicillin-susceptible strains of *S. aureus*, with a reported incidence of 13% in a recent European study [19]. Another gene, msrB from *Staphylococcus xylosus*, which is nearly identical to the 3′ end of msr(A), has been reclassified as msr(A) [3]. It contains a single ATP-binding domain but also confers an MSB phenotype.

The msr(A) gene has not been found in streptococci. In the genus *Streptococcus*, mef(A) genes encode an efflux pump, which can be found in clinical isolates of *S. pneumoniae* and *S. pyogenes*, in other species of streptococci (oral streptococci, group C and G streptococci, and *Streptococcus agalactiae*), and in enterococci. The original mef(A) gene was reported in *S.
Table 3. Macrolide resistance among Streptococcus pyogenes isolates by region or country.

<table>
<thead>
<tr>
<th>Country (city)</th>
<th>Year of study</th>
<th>Streptococcus pyogenes isolates</th>
<th>Resistant to erythromycin, %</th>
<th>With M phenotype, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1994–97</td>
<td>2.6</td>
<td>NS</td>
<td>[62]</td>
<td></td>
</tr>
<tr>
<td>USA (San Francisco)</td>
<td>1994–95</td>
<td>32^a, 9^b</td>
<td>NS</td>
<td>[63]</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>1998</td>
<td>4.6^c</td>
<td>21.7</td>
<td>[64]</td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>1990–93</td>
<td>0</td>
<td>0</td>
<td>[65]</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>1994–98</td>
<td>6.9</td>
<td>87.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1993–97</td>
<td>6.5</td>
<td>84</td>
<td>[66]</td>
<td></td>
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<tr>
<td>Finland</td>
<td>1992</td>
<td>16.5</td>
<td>NS</td>
<td>[67]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>19</td>
<td>NS</td>
<td></td>
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<td>1994</td>
<td>15.6</td>
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<td></td>
</tr>
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<td></td>
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<td>NS</td>
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<td></td>
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<td></td>
<td>1996</td>
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<tr>
<td>France</td>
<td>1996–99</td>
<td>6.2</td>
<td>45.2</td>
<td>[68]</td>
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<tr>
<td>Italy</td>
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<td>NS</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>26.8^a</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>1996</td>
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<td>NS</td>
<td>[61]</td>
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<tr>
<td></td>
<td>1993</td>
<td>5.8</td>
<td>NS</td>
<td></td>
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<td></td>
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<td>NS</td>
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<td>1998</td>
<td>23.5</td>
<td>95.6</td>
<td>[69]</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>1994</td>
<td>2</td>
<td>NS</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>41.3</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>Taiwan</td>
<td>1992–98</td>
<td>63.2</td>
<td>64.3</td>
<td>[71]</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** All isolates with the M phenotype (resistance to erythromycin, susceptibility to clindamycin without antagonism) are erythromycin-resistant strains; NS, not shown in the publication.

^a Invasive strains.
^b Throat isolates.
^c Range, 0–9.4%.
^d Range, 0–19.1%.
^e Range, 13–62%.

pyogenes [20]. A similar gene, once called mef(E), but now reclassified as mef(A), was reported later in S. pneumoniae [21]. The Mef(A) protein belongs to the MFS family and spans the membrane 12 times. The efflux is driven by the proton motive force and affects only 14- and 15-membered ring macrolides (M phenotype). There is no resistance to 16-membered ring macrolides, clindamycin, or streptogramin B, even after in-

**DRUG MODIFICATION**

Inherent to this mechanism of resistance, and unlike target modification, inactivation of antibiotics confers resistance to structurally related antibiotics only. Esterases and phosphotransferases reported in enterobacteria confer resistance to erythromycin and other 14- and 15-membered macrolides but not to lincosamides. So far, these resistances have not been considered of major clinical importance, because enterobacteria are not targets for macrolides, apart from the particular use of oral erythromycin for selective decontamination of the digestive tract. More worrisome is the finding of clinical isolates of S. aureus producing phosphotransferases encoded by mph(C) genes, although only a few strains have been reported to date [25]. Lincosamide nucleotidyltransferases encoded by Inu(A) (formerly linA) and Inu(B) (formerly linB) genes in staphylococci (S. aureus and coagulase-negative staphylococci) and Enterococcus faecium, respectively, inactivate lincosamides only [3]. Both genes confer frank resistance to lincomycin, but clindamycin remains active, with MICs that are increased by only 1 or 2 dilutions [26, 27]. However, the bactericidal activity of clindamycin, which is already weak against susceptible strains, is totally abolished [26]. Because of dissociated resistance among lincosamides, detection of this phenotype is possible only if lincomycin, instead of clindamycin, is tested. The impact of the in vitro alteration of clindamycin activity on the therapeutic efficacy of the drug is unknown. In addition, this resistance is rare in S. aureus (having been found in <1% of the strains) but is more frequent in coagulase-negative staphylococci (estimated frequency 1%–7% of strains), depending on the staphylococcal species [26]. The Inu(B) gene was detected in 10% of E. faecium strains in 1 study, but its expression was masked by the coexistence of erm in all strains [27].

On the whole, although a panel of genes is able to inactivate macrolides and lincosamides, their presence in gram-positive cocci has not turned out to be a success story for bacteria. This could be the result of (1) a weak clinical impact caused by the low-level of resistance conferred to erythromycin, by mph(C)
**Figure 1.** Detection of resistance to macrolides and lincosamides in staphylococci. Resistance phenotypes were identified on the basis of erythromycin- and clindamycin-or lincomycin-susceptibility tests and on the basis of clinical implications. L, lincosamides; MS B, macrolides and streptogramins B; Md, membered; R, resistant; S, susceptible.

When present alone and to clindamycin, by the \textit{lnu} genes, or (2) of a failure of detection.

### IMPACT OF MACROLIDE RESISTANCE ON PATIENT OUTCOME

There is controversy concerning the clinical relevance of in vitro macrolide resistance, because few patients with clinical failure and resistant strains have been reported [28, 29]. Certain authors have attributed this paradox to the ability of newer macrolides—in particular, azithromycin—to reach high concentrations in the infected tissues [28]. It should be stressed, however, that newer macrolides are, in fact, concentrated in the phagocytic cells rather than in the extracellular fluids [30]. Because \textit{S. pneumoniae} and \textit{S. pyogenes} are thought to be primarily extracellular pathogens, extracellular drug concentrations should be considered as being predictive of therapeutic success. Internalization of a few \textit{S. pyogenes} organisms, however, might contribute to the building of a reservoir of persisting bacteria that escape penicillins that do not enter eukaryotic cells or macrolides when the strains are resistant to these antimicrobials [31]. A recent study has shown that Italian erythromycin-resistant \textit{S. pyogenes} has a greater ability to be internalized in human cells than does the erythromycin-susceptible strains, possibly leading to difficulties in eradication [31].

In fact, the frequent use of macrolides for nonsevere infections that often have a spontaneous favorable evolution makes it difficult to establish the correlation between the in vitro resistance of microorganisms to macrolides and the clinical outcome of patients treated with these antibiotics. Therefore, to reach firm conclusions, studies based on clinical evaluation should include a large number of patients infected with macrolide-resistant microorganisms and treated with these antibiotics. For these reasons, bacterial eradication is often used as an evaluation criterion. The question of the accuracy and clinical relevance of this criterion has been addressed in few studies and is not completely solved [29]. Clear correlation between eradication of bacteria on days 4–5 and clinical outcome was, however, shown in children with acute otitis media due to \textit{S. pneumoniae} [29, 32]. The impact of macrolide resistance on bacterial eradication has been evaluated in children with acute otitis media or tonsillitis and in adults with pneumonia. In a study comparing the efficacies of azithromycin and cefaclor in young children with acute otitis media, Dagan et al. [33] found that pneumococcal eradication by azithromycin was achieved at day 4 or 5 in a group of 12 patients infected with strains for which the MIC of the antibiotic was \(\leq 0.06\) \(\mu\)g/mL. By contrast, pneumococcal strains with an MIC of azithromycin \(\geq 32\ \mu\)g/mL persisted in all 5 patients initially infected with resistant strains, whereas 1 patient acquired a resistant strain
Figure 2. Detection of resistance to macrolides and lincosamides in streptococci. Resistance phenotypes were identified on the basis of erythromycin- and clindamycin-susceptibility tests and on the basis of clinical implications. I/R, intermediate/resistant; MSB, macrolides and streptogramins B; Md, membered; S, susceptible.

during treatment. Although the genetic content of the strains had not been studied, the high level of resistance to macrolides suggested that resistance was due to \textit{erm} genes. In another study, the review of the records of 41 patients with pneumococcal bacteremia revealed that 7 had previously received antibiotic therapy [34]. Three of the patients did not appear to have true antibiotic therapy failure, whereas the 4 remaining patients had previously been treated with azithromycin or clarithromycin for 3–5 days. The 4 blood isolates had an M phenotype and a moderate level of resistance to macrolides, with MICs of erythromycin equal to 8 \(\mu\text{g/mL}\) or 16 \(\mu\text{g/mL}\).

The data from the studies of acute pharyngitis are even more difficult to analyze, because recolonization or spontaneous clearance of \textit{S. pyogenes} in the throat appears to occur frequently. Varaldo et al. [35] failed to find a correlation between in vitro resistance to macrolides and noneradication in children with pharyngotonsilitis due to \textit{S. pyogenes} who were receiving macrolide therapy. In another study, the rate of eradication of \textit{S. pyogenes} in patients who had pharyngitis and who were treated with clarithromycin did not differ significantly from that in patients with erythromycin-resistant strains or those in patients with erythromycin-susceptible strains, despite a trend toward a higher rate of eradication in the latter group [36]. Of note, none of the 6 patients with strains highly resistant to erythromycin were cured. Failure of erythromycin could be demonstrated by Seppälä et al. [37], who found that this antibiotic significantly failed to cure 9 (47%) of 19 patients infected with erythromycin-resistant group A streptococci (including moderately resistant strains), as compared with 1 (4%) of 26 patients with erythromycin-susceptible isolates. Taken together, these studies tend to suggest a correlation between macrolide resistance, at least when expressed at a high level, and clinical failure. Because of differences in pharmacokinetics, all macrolides probably are not equivalent in their ability to eradicate strains with low-level macrolide resistance. Finally, more studies that include homogeneous groups of patients are needed to confirm the in vivo impact of in vitro resistance—in particular, that caused by drug efflux—and to propose breakpoints that are predictive of clinical failure or success.

**INCIDENCE OF MACROLIDE RESISTANCE**

Table 2 provides data on the incidence of macrolide resistance in \textit{S. pneumoniae} published for <2 years. Huge geographic differences, from 3% to 74%, are observed in the resistance frequencies reported for individual countries. In addition, considerable variations can be seen within a country, depending on the source of the strains (teaching/nonteaching hospital, or community), patient age, sample origin, seasonal factors, and pneumococcal serotype [39, 58]. Similar to the case of penicillin resistance, higher prevalence of macrolide resistance is generally seen among children and for pneumococci from middle ear fluids. These differences have obvious impact on the therapeutic efficacy of agents of the MLS class. Although useful for analysis global trends toward resistance, nationwide surveys do not always take into account these parameters, and there is a need
for physicians to be aware of local resistance patterns according to patient age and type of infection. In general, the higher the rate of penicillin resistance, the higher the rate of macrolide resistance. In a large study that involved hospitals from 21 countries around the world, macrolide resistance was detected in 12.8% of penicillin-susceptible pneumococci versus 55.7% of penicillin-resistant strains [59]. In another recent study [42], similar variations between penicillin-susceptible and -resistant strains (4% and 61% in the United States, 4% and 27% in Latin America, 10% and 52% in Europe, and 22% and 76% in Asia, respectively), were found. This relationship is especially marked in the United States (table 2) and is also shown for trimethoprim-sulfamethoxazole resistance. There are, however, some exceptions. For instance, in Italy, the low rate of penicillin resistance contrasts with the high rate of erythromycin resistance (table 2).

No substantial difference can be observed between percentages of resistance to azithromycin, clarithromycin, and erythromycin, which confirms cross-resistance between the 3 antimicrobials [42, 44, 52]. By contrast, in several countries, the incidence of clindamycin resistance may be much lower than that of erythromycin resistance. The spread of erythromycin-resistant strains that harbor the mef(A) gene accounts for this difference. This holds true especially in the United States, in contrast to most European countries, where erm(B)-containing strains are widespread. The reasons for these differences are unexplained. Longitudinal studies showed that the incidence of macrolide resistance, which was 10%–16% in 1994, doubled in 1999 in several areas of the United States [41, 58]. This marked increase was, for the most part, related to the emergence of mef(A)-containing strains [41]. Several European countries (i.e., Finland, Italy, and Spain) faced an increase in erythromycin resistance in S. pyogenes at the beginning of the 1990s [37, 60, 61] (table 3). Recent data show that very high frequencies of macrolide resistance are reported in Asia, whereas, to date, resistance does not seem to be a problem in the United States (table 3); however, particular high frequencies can be seen, as exemplified by a 32% rate of erythromycin resistance in S. pyogenes collected from invasive disease–related specimens in San Francisco County [63]. In the vast majority of S. pyogenes strains, erythromycin resistance is caused by the presence of the efflux gene mef(A) or the methylase genes erm(TR) (erm(A)) and erm(B). The mef(A) and erm(TR) genes are frequently predominant [24, 64].

In several studies, investigators have tried to establish connections between consumption of macrolides and widespread resistance. Frequent coresistance to several antibiotics in pneumococci makes evaluation of the impact of a specific class of antibiotic on resistance problematic. This is not the case for S. pyogenes, which remains susceptible to penicillins. The most convincing data, from Finland, were high rates of macrolide resistance in S. pyogenes, along with an increase in macrolide consumption and a subsequent decrease after significant reduction in the use of macrolides in outpatients [67]. Similarly, an increase in macrolide resistance in Spain since 1995 was related to the increase in consumption of macrolides, especially those that are taken once or twice daily [61]. This relationship was not found in a Canadian study [64], however, and temporal relations do not prove causality. To explain differences between countries with regard to sudden emergences of resistance, Granizo et al. [61] have hypothesized that spread of resistance occurs when macrolide consumption exceeds a critical threshold of selective pressure. It is remarkable that, in intracellular pathogens, such as Legionella, Chlamydia, and Mycoplasma species, findings of resistance remain anecdotal.

CONCLUSIONS

The multiplicity of mechanisms that confer resistance to macrolides is reflected by the complexity of the resistance phenotypes; however, the most clinically important and widespread determinants in gram-positive organisms are the methylase and efflux genes. Identification of the resistance mechanisms is important with regard to the use of clindamycin and 16-membered ring macrolides. The double-disk diffusion technique with erythromycin and lincomycin (or clindamycin) is useful to guide interpretation of the susceptibility test [72] (figures 1 and 2). The incidence of resistance is highly variable with regard to the country and, most importantly, the patterns of infections observed among patients. For this reason, local statistics are of crucial value for empiric therapy. Surveillance of both the incidence of macrolide resistance and the respective prevalence of the various resistance mechanisms is justified by the rapid variations in macrolide resistance observed in several countries.

Acknowledgment

I wish to thank Patrice Courvalin for reading the manuscript.

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